LOCATION OF THE SUPERFICIAL EPITHELIAL BARRIER TO SKIN PENETRATION* †

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The present study was undertaken to determine as precisely as possible the location of the barrier in the skin to the passage of selected substances. Topical anesthetics, antihistaminics and histamine were chosen as indicators because of their clinical significance. Other workers have contributed to this field and have defined the problem with which this study deals.

Various theories have been proposed of the location and the cause of the barrier. Rein (1) stated that electrolytes cannot pass through a single layer between cornified and non-cornified epidermis ("stratum lucidum") because this layer is impermeable to ions. Rothman (2) agreed and proposed that the "transitional layers between cornified and non-cornified epidermis" should be regarded as the site of impermeability for electrolytes.

Rothman (2) also claims that the role of the horny layer has been misinterpreted as a barrier to percutaneous absorption. He states that the layer has large pores and is permeated even by large molecular aggregates. He considers the lowest compact layer of the horny layer to be different from the looser strata above and believes that the lowest layer, consisting of a thickness of five to six cell layers, is a part of the epithelial barrier. MacKee (3), *et al.* describe the barrier in man as including "the lowest portion of the stratum corneum plus the stratum lucidum and stratum granulosum."

Wolf (4), in 1940, described a method of removing the stratum corneum in layers, using cellophane adhesive (Scotch tape®) for the purpose. Pinkus (5) revived interest in this procedure and used it to study the reaction of the stratum mucosum to such stripping. Lorincz (6) has lately devised a machine to accomplish the same purpose. It usually requires 25 to 30 strippings to remove the entire stratum corneum from the skin of the anterior surface of the arm or forearm. Lorincz found that, after the stratum corneum's removal, the epithelium became easily permeable to aqueous solutions of such agents as pitressin, adrenalin and histamine in concentrations which ordinarily have no effect on the intact skin. Evidently the epithelial barrier is within the stratum corneum.

MATERIALS AND METHODS

The normal skin of the volar surface of the forearm was used in all experiments to be reported, except those in which the vermilion border of the lip, the buccal mucosa, the anterior nares and the anterior aspect of the thigh were chosen for

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special studies. Selected levels of the epithelium were exposed by stripping off cells of the stratum corneum with cellophane adhesive tape (Scotch tape). The tape was repeatedly applied firmly and removed briskly in the same area of skin, usually in increments of five strippings between tests. The procedures were performed initially upon the author and in all cases were repeated on several additional subjects, most of whom were trained medical observers.

Biopsies of the exact sites stripped and tested physiologically were performed with a dermal biopsy punch.

Test solutions were prepared freshly. The water soluble salts were dissolved in distilled water. The water insoluble bases were dissolved in a solvent composed of 45 per cent isopropyl alcohol, 10 per cent glycerine and 45 per cent water. The presence of anesthesia was determined by subjective response to pinprick.

In some of the experiments, anesthesia was produced by applying solutions of the base or salt under an occlusive dressing in the manner of a patch test. Testing for anesthesia was done at frequent intervals. If no anesthesia was obtained in 30 minutes, the absorbent cotton was remoistened with the anesthetic solution and reapplied. In other experiments, the area to be tested was surrounded by a wall of petrolatum about 2 mm. high. The solution was then placed in the well thus formed and tested for anesthesia at regular intervals. This procedure produced continuous intimate contact without the use of an occlusive dressing.

The experiments with histamine dihydrochloride were performed under occlusive dressings.

RESULTS

There are, of course, variations in the thickness of the stratum corneum on different parts of the body and on the same part in different individuals. In the same person, the differences between various areas of the anterior surfaces of the arms and forearms is minimal except for a more marked thinness in and near the antecubital fossa and on the medial aspect of the arm. The palms and soles have a very thick, cornified layer, the removal of which requires hundreds of strippings.

One must also remember that the amount of stratum corneum removed with each stripping is not constant. It will vary with not only the location of the area

TABLE 1

Anesthetic	Number of times stripped	Time required for anesthesia
2% Xylocaine base	10	1 min.
5% Xylocaine base	5	3 min.
1% Xylocaine HCl	10	4 min.
2% Xylocaine HCl	10	1 min.
1% Pyribenzamine base	10	2 min.
2% Pyribenzamine base	15	2 min.
1% Pyribenzamine HCl	15	4 min.
2% Pontocaine base	15	4 min.
2% Pontocaine HCl	15	3 min.

stripped but also with the variability of the adhesive, the degree of moistness of the skin and the amount of lipid soluble material on the skin. For these reasons the term "5 strippings" does not always indicate the exact, but only the approximate, amount of stratum corneum removed. The variation is not very great, however, since approximately the same results are obtained on repeated strippings.

It was surprising to find that the removal of approximately the outer half of the stratum corneum resulted in an increase in skin penetrability. For example, 2 per cent aqueous solution of Xylocaine® hydrochloride, Pyribenzamine® hydrochloride or Pontocaine® hydrochloride do not produce anesthesia when swabbed upon the intact skin. A similar lack of anesthesia results from the application of 2 per cent concentrations in dilute alcohol of the bases of the above substances. When, however, the skin is stripped only 10 or 15 times, it becomes penetrable to both the above types of solution, although complete removal of the stratum corneum usually requires an additional 10 to 20 strippings with Scotch tape. Thus, after removal of approximately one-half to two-thirds of the stratum corneum, application of the above substances results in anesthesia within a few minutes, as shown in Table 1.

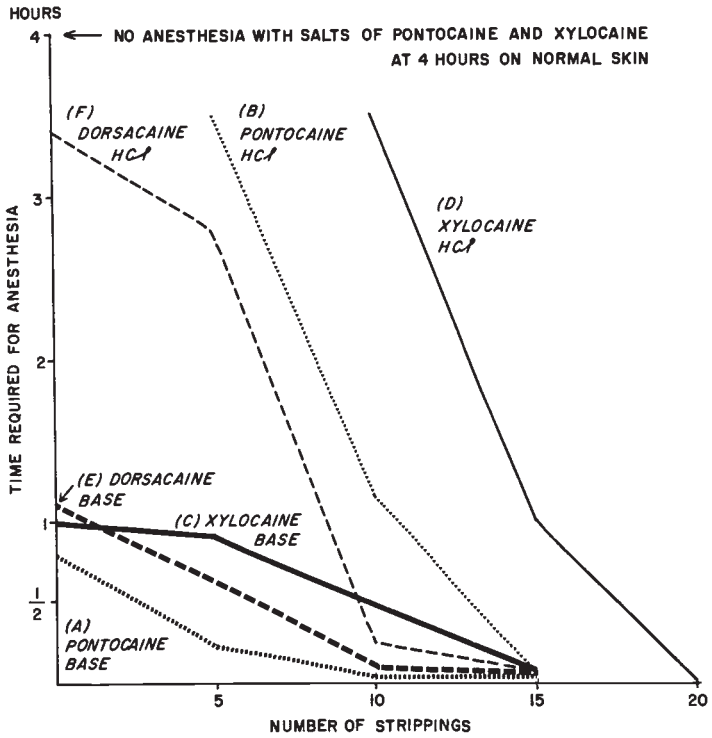

FIG. 1. Time required for anesthesia after application of anesthetic solutions under occlusive dressing on normal and stripped skin. (A) 2% Pontocaine Base in 45% alcohol; (B) 2% Pontocaine HCl in 45% alcohol; (C) 2% Xylocaine Base in 45% alcohol; (D) 2% Xylocaine HCl in 45% alcohol; (E) 2% Dorsacaine Base in 45% alcohol; (F) 2% Dorsacaine HCl in water.

The results obtained with occlusive dressings over cotton soaked with 2 per cent solutions of various anesthetic bases and salts show a progressive diminution in the time required to produce anesthesia with increase in the number of strippings (Fig. 1). On normal skin, application of Pontocaine® hydrochloride and Xylocaine® hydrochloride solutions failed to produce anesthesia in four hours, and Dorsacaine® hydrochloride required three and one-half hours for anesthesia. The bases of all three produced anesthesia in from three-fourths to one and one-third hours on normal skin. The time required for anesthesia with both salts and bases diminished rapidly with increased number of strippings, more quickly with the salts, and more gradually with the bases. After from 10 to 20 strippings, the time required in both cases diminished to a few minutes.

Similar experiments, using occlusive dressings with an aqueous solution of histamine dihydrochloride containing 1 per cent histamine, calculated as histamine base, resulted in a progressive diminution in the time required to produce itching and whealing with increase in the number of strippings (Fig. 2).

During the experiments with occlusive dressings, it was necessary to remove the dressings at regular intervals for testing. With experiments lasting 3 to 4 hours, the absorbent cotton tended to become dry and did not always establish complete contact with the underlying skin. As a result, the time required for anesthesia with solutions of the anesthetic salts was longer than was really necessary. This is shown by the experiments in which a well of the anesthetic salt solution was allowed to remain in contact with the skin until it became

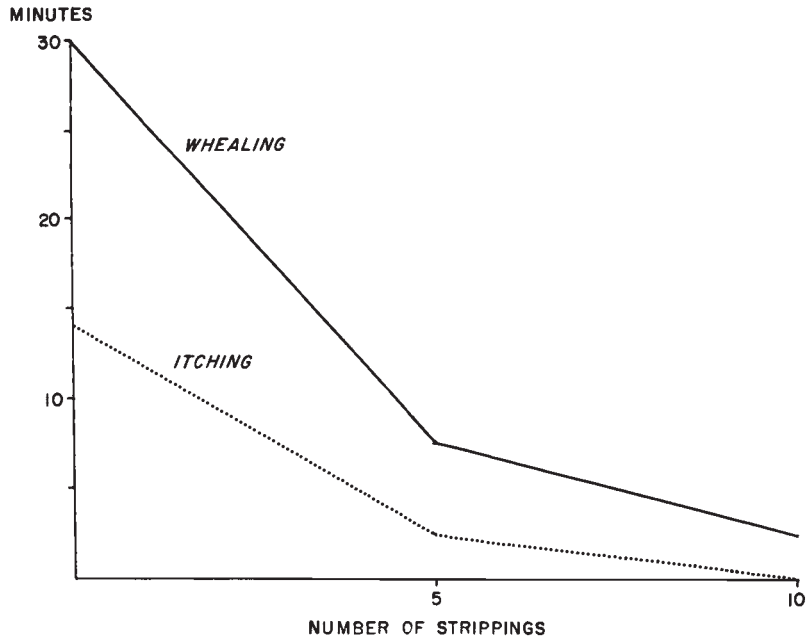

FIG. 2. Reaction of normal and stripped skin to histamine dihydrochloride solution in concentration equivalent to 1% histamine base, showing time of onset of whealing and itching.

TABLE 2

Number of strippings	Concentration of histamine	Effect			
		Erythema	Wheal	Flare	Itch
0	1:5,000	0	0	0	0
5	1:5,000	0	0	0	0
10	1:5,000	0	0	0	0
15	1:5,000	+	+	0	±
20	1:5,000	+	+	0	+
25	1:5,000	+	+	+	+

TABLE 3

Region tested	Anesthetic	Effect
Oral mucosa of cheek	2% Pontocaine® HCl	Anesthesia in 5 min.
Oral mucosa of lower lip	2% Pontocaine® HCl	Anesthesia in 4 min.
Vermilion border of lip	2% Pontocaine® HCl	Anesthesia in 9 min.
Anterior portion of nasal septum	2% Pontocaine® HCl	Anesthesia in 12 min.
Forearm after stripping 15 times	2% Pontocaine® HCl	Anesthesia in 3 min.

anesthetic (Fig. 4). Two per cent Dorsacaine® HCl and 2 per cent Pontocaine® HCl solutions in water produced anesthesia after a two-hour application, and 2 per cent Xylocaine® HCl solution required 15 minutes longer. After the skin had been stripped 5 to 15 times there was a decided drop in the time required to produce anesthesia; after 15 strippings the drop became much slower, tending toward the horizontal, reaching a line, after 25 strippings, where only one or two minutes were required to produce anesthesia.

RESULTS WITH TOPICAL HISTAMINE PHOSPHATE

Histamine phosphate in concentrations equivalent to 1:5,000 of the base, when applied to intact skin, fails to produce a detectable effect. On the forearm 10 strippings failed to remove the barrier, but after 15 strippings the histamine produced recognizable changes. Typical results are recorded in Table 2. It is noteworthy that each of the visible changes, erythema, wheal, and flare, and the subjective sensation of itch, did not occur simultaneously at the critical levels of the experiment and with the concentrations of histamine used.

Topical anesthetics have long been known to anesthetize mucous membranes with ease, whereas they had little effect on intact skin. A comparison of the rapidity with which anesthesia appears in various mucosal and transitional epithelia and stripped skin is presented in Table 3. The areas without a distinct organized outer horny layer also lack an effective barrier to the penetration of electrolytes as represented by a topical anesthetic.

HISTOLOGIC OBSERVATIONS

The progressive removal of the stratum corneum by stripping is illustrated in the photomicrographs of biopsies made at intervals of 5 strippings (Fig. 3

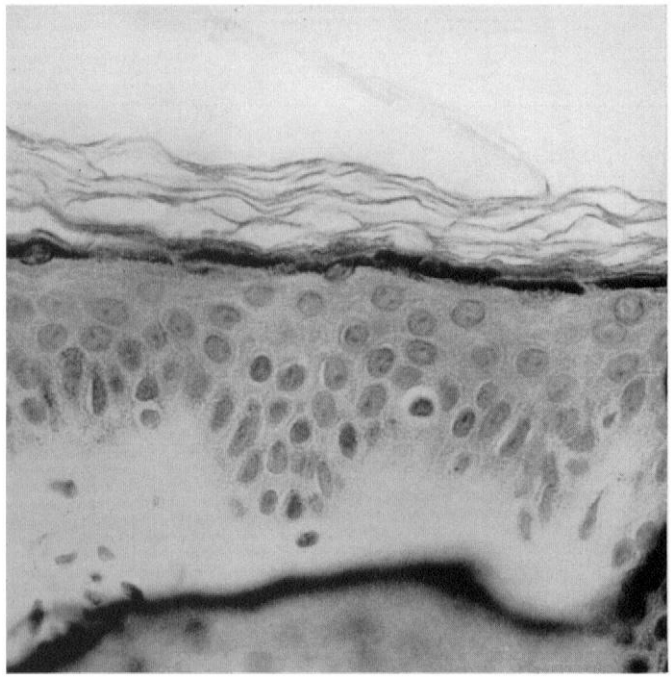

FIG. 3 (A)

FIG. 3. (A) Normal skin before stripping; (B) After 5 strippings; (C) After 10 strippings; (D) After 15 strippings; (E) After 20 strippings.

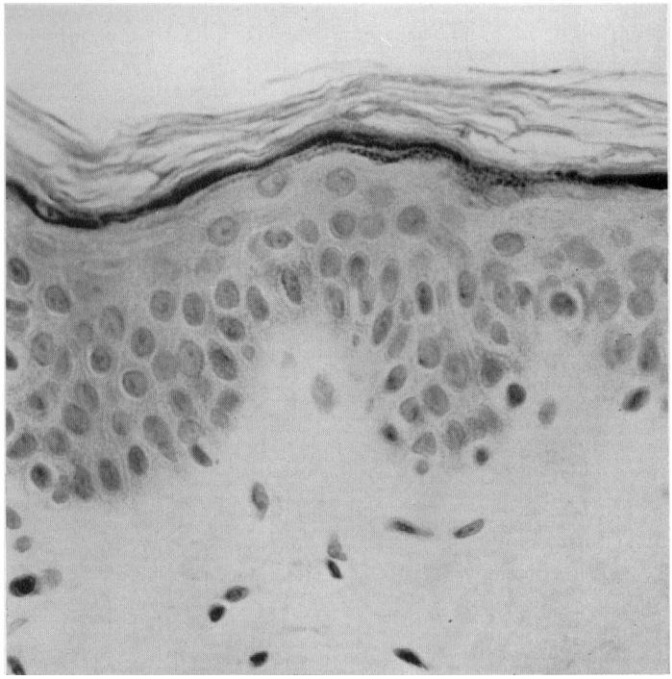

FIG. 3 (B)

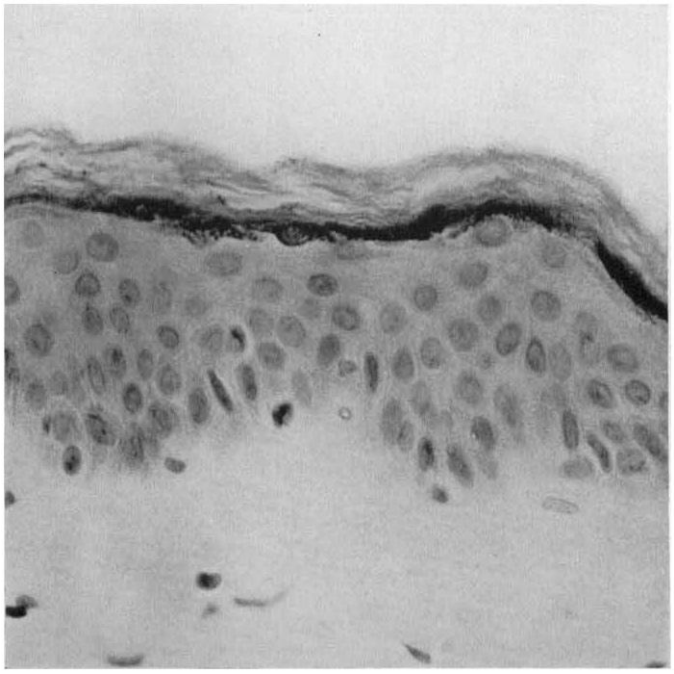

FIG. 3 (C)

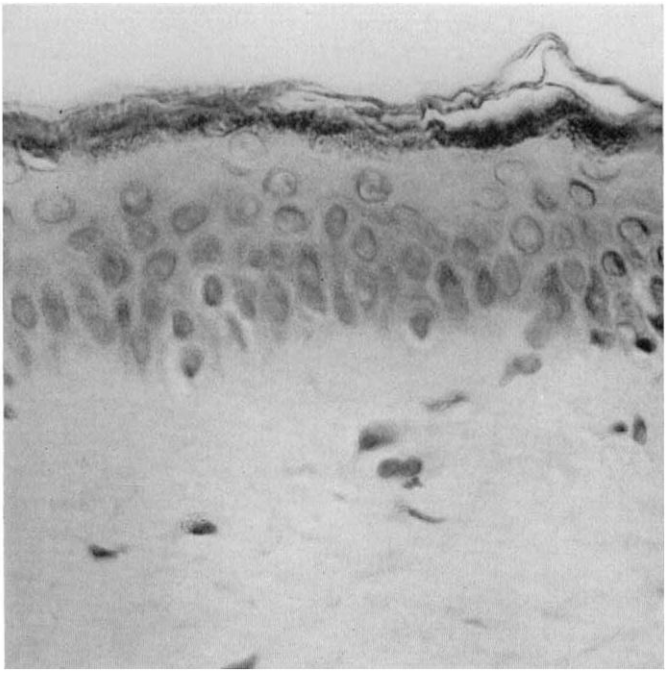

FIG. 3(D)

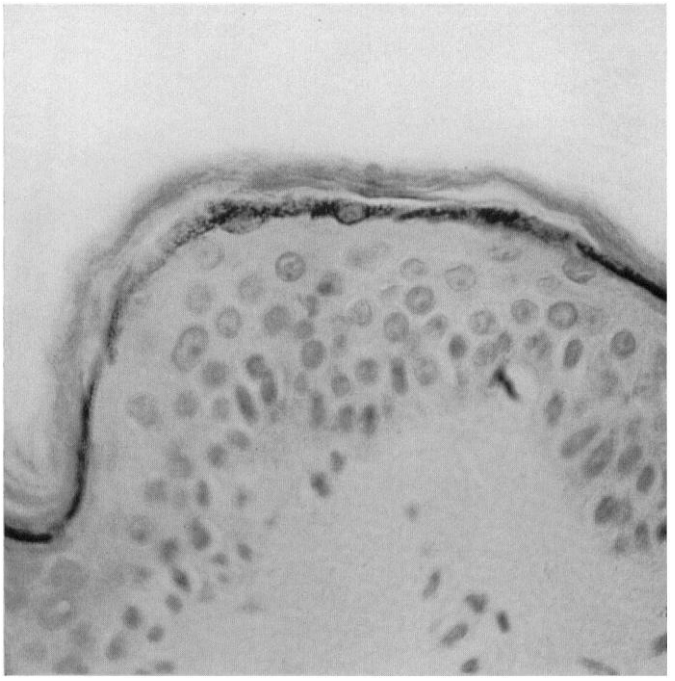

FIG. 3(E)

A–D). These histologic sections show clearly that the lowermost part of the stratum corneum remains after 15 or 20 strippings.

DISCUSSION

It is evident from the above experiments that practically the entire thickness of the stratum corneum participates in the formation of the epithelial barrier to percutaneous absorption of electrolytes. One may except the outermost layer or two of stratified cells which have begun to loosen from the rest of the stratum corneum prior to exfoliation. This outermost layer permits penetration as shown by the easy staining of the surface to dyes. One may also except the innermost layer or two of stratum corneum cells which have just been formed from the stratum granulosum and have not yet developed resistance to penetration.

A comparison of the time required to produce anesthesia on normal skin and on skin stripped 15 times (Fig. 1), shows a diminution with 2 per cent Dorsacaine® base solution, 2 per cent Xylocaine® base solution and 2 per cent Pontocaine® base solution, of one hour and ten minutes, fifty-five minutes, and forty-five minutes, respectively. With 5 additional strippings, the further diminution in time is negligible; amounting to either nothing or five minutes at the most. The outer 15 strippings therefore form the principal barrier to the penetration of the 2 per cent base solutions. Similarly, with the salt solutions (Fig. 4), the diminution in time required to produce anesthesia with 2 per cent Dorsacaine® HCl solution on normal skin from that on skin stripped 15 times was two hours;

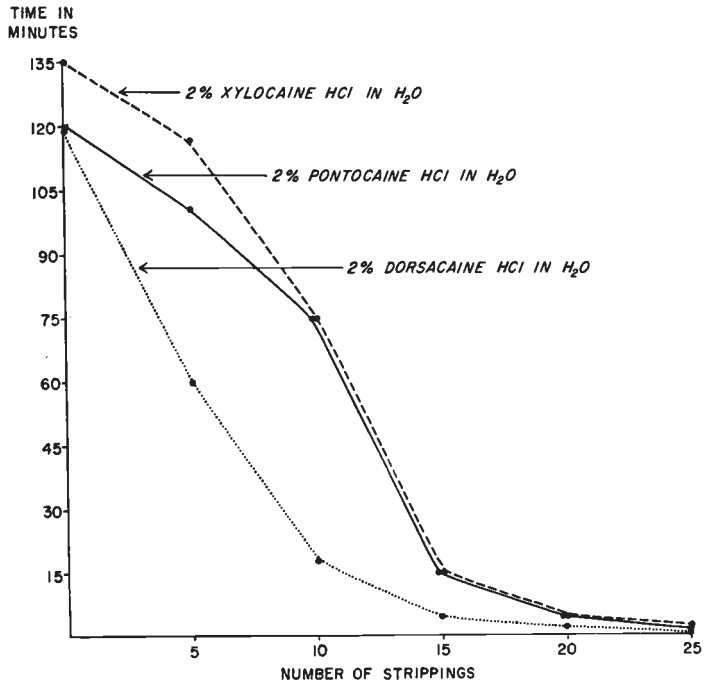

FIG. 4. Time required for anesthesia after continuous application of anesthetic solutions in a well formed by a wall of petrolatum on normal and stripped skin.

a further stripping of 5 times reduced the time required for anesthesia by only a few minutes. Similar results were obtained with 2 per cent Pontocaine® HCl and 2 per cent Xylocaine® HCl solutions. The above results show that stripping the skin 15 times, thus removing approximately one-half to two-thirds of the outer stratum corneum, also removes the main barrier to penetration of the anesthetics used. The remaining stratum corneum, which includes the barrier isolated by Szakall (7), offers very little resistance to the passage of the anesthetic solutions. The experiments with histamine phosphate solution (Fig. 2) similarly show that 10 strippings of stratum corneum have removed the principal barrier to histamine penetration.

The barrier to anesthetic solutions on the palms and soles obviously differs from that elsewhere because of the very thick stratum corneum. It requires about 600 strippings with Scotch tape to remove the stratum corneum to the point where it becomes easily penetrable to anesthetic salts or bases. The amount of stratum corneum removed to accomplish this constitutes about 98 per cent or more of its thickness. On the soles and palms, therefore, the barrier is much thicker than elsewhere and also consists of practically the entire thickness of the stratum corneum.

SUMMARY

The superficial barrier to percutaneous penetration of the substances studied is formed by practically the entire thickness of the stratum corneum. The greater

part of this barrier is formed by the outer half to two-thirds of the stratum corneum.

I wish to thank Dr. Harvey Blank for many suggestions while carrying on this work. Thanks are also due to the Winthrop Laboratories for supplies of Pontocaine®, the Astra Pharmaceutical Products for Xylocaine®, the Ciba Pharmaceutical Company for Pyribenzamine®, and the Smith-Dorsey Company for Dorsacaine®.

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